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Abstract: Background: The HIV drugs lopinavir and ritonavir have recently been reported to cause transient adrenal insufficiency in preterm newborns. We, therefore, considered HIV drugs as a cause of transiently elevated 17-hydroxyprogesterone (17OHP) levels in a neonatal screening test for congenital adrenal hyperplasia in a preterm girl exposed to zidovudine, efavirenz, tenofovir, and emtricitabine. Objective: So far, HIV drugs have not been tested for their effect on steroidogenesis and the steroidogenic enzyme activity of CYP21A2 specifically in an in vitro system. Methods: We tested the effect of efavirenz, tenofovir, emtricitabine, and zidovudine on steroidogenesis of human adrenal H295R cells. Cells were treated with the drugs at different concentrations including concentrations in therapeutic use. The effect on CYP21A2 activity was assessed by testing the conversion of radiolabeled 17OHP to 11-deoxycortisol. Cell viability was tested by an MTT assay. In addition, recombinant human CYP21A2 protein was used to assess direct drug effects on CYP21A2 activity. Results: We observed significantly decreased CYP21A2 activity in both in vitro testing systems after treatment with efavirenz at therapeutic concentrations. Moreover, efavirenz affected cell viability. By contrast, the other test drugs did not affect steroidogenesis. Follow-up of our patient revealed elevated 17OHP and androgen levels during the first weeks of life, but values normalized spontaneously. Genetic testing for CYP21A2 mutations was negative. Thus, it remains unsettled whether the transient 17OHP elevation in this baby was due to a drug effect. Conclusion: The HIV drug efavirenz inhibits CYP21A2 activity in vitro through direct interaction with enzyme catalysis at therapeutic concentrations. This may have clinical implications for HIV treatment in children and adults. However, so far, clinical data are scarce, and further studies are needed to be able to draw clinical conclusions.

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HIV Drug Efavirenz Inhibits CYP21A2 Activity with Possible Clinical Implications

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Keywords

HIV treatment · Efavirenz · 21-Hydroxylase deficiency · Androgens · Adrenal insufficiency · Neonatal screening · Congenital adrenal hyperplasia

Abstract

Background: The HIV drugs lopinavir and ritonavir have recently been reported to cause transient adrenal insufficiency in preterm newborns. We, therefore, considered HIV drugs as a cause of transiently elevated 17-hydroxyprogesterone (17OHP) levels in a neonatal screening test for congenital adrenal hyperplasia in a preterm girl exposed to zidovudine, efavirenz, tenofovir, and emtricitabine. ***Objective:*** So far, HIV drugs have not been tested for their effect on steroidogenesis and the steroidogenic enzyme activity of CYP21A2 specifically in an in vitro system. ***Methods:*** We tested the effect of efavirenz, tenofovir, emtricitabine, and zidovudine on steroidogenesis of human adrenal H295R cells. Cells were treated with the drugs at different concentrations including concentrations in therapeutic use. The effect on CYP21A2 activity was assessed by testing the conversion of radiolabeled 17OHP to 11-deoxycortisol. Cell viability was tested by an MTT assay. In addition, recombinant human CYP21A2 protein was used to assess direct drug effects on CYP21A2 activity. ***Results:*** We observed significantly decreased CYP21A2 activity in both in vitro testing systems after treatment with efavirenz at therapeutic concentrations. Moreover, efavirenz affected cell viability. By contrast, the other test drugs did not affect steroidogenesis. Follow-up of our patient revealed elevated 17OHP and androgen levels during the first weeks of life, but values normalized spontaneously. Genetic testing for *CYP21A2* mutations was negative. Thus, it remains unsettled whether the transient 17OHP elevation in this baby was due to a drug effect. ***Conclusion:*** The HIV drug efavirenz inhibits CYP21A2 activity in vitro through direct interaction with enzyme catalysis at therapeutic concentrations. This may have clinical implications for HIV treatment in children and adults. However, so far, clinical data are scarce, and further studies are needed to be able to draw clinical conclusions.

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Introduction

Adrenal insufficiency (AI) may be a life-threatening condition due to inadequate production of glucocorticoids and mineralocorticoids. It is classified as primary AI (underlying cause in the adrenals) and as secondary AI (underlying defect in the hypothalamic-pituitary control of the adrenals). In newborns, congenital adrenal hyperplasia (CAH) due to genetic mutations in the *CYP21A2* gene is the leading cause of primary AI and affects about 1 in 10,000–15,000 newborns in Europe [1–3]. CYP21A2 is essential for both mineralocorticoid and glucocorticoid production because it mediates the 21-hydroxylation of progesterone to deoxycorticosterone and 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol (11DOC) [2]. The severe, classic, salt-wasting form of CYP21A2 deficiency is a potentially life-threatening condition characterized by low or absent production of cortisol and aldosterone as well as overproduction of adrenal androgens, and manifests early in life [1–4]. The milder, non-classic form of CAH may manifest later in life, and predominantly results in adrenal androgen excess. While girls with classic CAH often present ambiguous genitalia at birth due to intrauterine overproduction of androgens [5], boys do not show physical signs suggestive of CAH [4, 6]. Therefore, many countries have introduced a neonatal screening program for early diagnosis of CAH based on blood 17OHP levels reflecting CYP21A2 enzyme deficiency [3, 6].

However, 17OHP blood screening by heel stick may reveal both false-positive and false-negative results. The interpretation of elevated levels in preterm [7] or stressed newborns [8] is especially challenging. Also, 17OHP may be elevated in other rare forms of CAH, such as HSD3B2, CYP11B2, or P450 oxidoreductase deficiencies, which also require prompt corticosteroid replacement therapy [9, 10]. Furthermore, the activities of adrenal enzymes can be influenced by drugs and toxins and thereby cause primary AI in rare cases. Drugs such as ketoconazole, metyrapone, etomidate, mitotane, and abiraterone are known inhibitors of steroid enzymes, including CYP11A1, CYP17A1, CYP21A2, and CYP11B1/2 [11, 12].

In recent clinical studies, it has been suggested that some HIV drugs may also affect steroidogenesis. Elevated serum 17OHP and dehydroepiandrosterone levels were found in neonates treated perinatally with the HIV-1 protease inhibitors lopinavir and ritonavir [13, 14]. Three premature babies presented with clinical and biochemical signs of adrenal dysfunction after postnatal treatment with these drugs [13], while a full-term neonate was noted to have high serum 17OHP and potassium levels after pre- and postnatal treatment with lopinavir and ritonavir [14]. In our clinic, we also followed a preterm baby of an HIV-infected mother for repeatedly high levels of 17OHP during neonatal screening. The drugs efavirenz, emtricitabine, and tenofovir (Atripla®) were prescribed to the mother during pregnancy to reduce the mother-to-child transmission of HIV, and the neonate was treated prophylactically with zidovudine postnatally. According to the current literature, these HIV drugs have not been tested for a possible effect on steroidogenesis.

In 2017, the World Health Organization reported that about 18 million women worldwide were infected with HIV. They also reported that HIV treatment during pregnancy was very effective and reduced the mother-to-child transmission of an HIV infection from 45 to 2% [15]. However, this means that a significant number of neonates are exposed to HIV drugs very early in life, and possible adverse effects of the drugs might be of significance.

Therefore, motivated by the clinical observations and stimulated by the fact that none of the HIV drugs in routine use has been tested for an effect on adrenal steroidogenesis, we tested some of these drugs for their effect on steroidogenesis, specifically regarding CYP21A2 activity in vitro.

Materials and Methods

Case Report

A 46,XX baby girl was born at 26 weeks of gestation after premature rupture of membranes to an HIV-infected mother. Birth weight was 760 g (P 25–50) and length 35 cm (P 50–75). Because of premature contractions, the mother received 2 doses of betamethasone at 23 5/7 weeks of gestation for induction of lung maturation. In addition, the mother was treated with the HIV drugs tenofovir, efavirenz, and emtricitabine (Atripla®) throughout pregnancy. The newborn was then treated with zidovudine for (post)exposure prophylaxis during the first 4 weeks of life (Fig. 1). The girl showed a normal physical exam at birth, including normal female external genitalia without signs of virilization, but she received a single dose of hydrocortisone (0.1 mg/kg) for low blood pressure. Initial laboratory workup revealed normoglycemia and normal electrolyte and 17OHP (80 nM; reference value for the gestational and postnatal age <192 nM) serum levels. However, while glucose and electrolyte levels remained normal, 17OHP increased to 292 nM on day 15 (normal level for gestational and postnatal age <141 nM) and was still elevated on day 19 (132 nM; normal <104 nM; DELFIA Neonatal 17αOH-progesterone kit, PerkinElmer, Switzerland), which prompted further workup for possible CAH. ACTH (8.3 ng/L; reference range 7.2–63.3) and cortisol (396 nM; reference range 171–536 nM) were both within the normal range at 3 weeks of age. Urine steroid profiling (gas chromatography-mass spectrometry) at 3 and 6 weeks of age revealed elevated pro-

gesterone and androgen metabolites (online suppl. Table 1; see www.karger.com/doi/10.1159/000500522 for all online suppl. material). Finally, serum 17OHP normalized after 4 weeks (Fig. 1), and the clinical course was completely unremarkable. The baby was discharged home at 36 1/7 weeks postconceptional age. She is currently 3 years old and healthy.

Urinary Steroid Profiling

Urinary steroid profiling was performed by an established, validated in-house method (gas chromatography-mass spectrometry) [16–19]. Measurements of spot urines (collected from cotton balls inserted in diapers) were normalized to creatinine (Quanti-Chrom Creatinine Assay; DICT-500, BioAssay Systems, Hayward, CA, USA). Accordingly, results are expressed in micrograms per millimole creatinine (online suppl. Table 1) as previously described for healthy neonates during the first year of life [16–18].

Genetic Analysis of the CYP21A2 Gene

Three fragments of the *CYP21A2* gene were amplified by PCR from genomic DNA using the CAH StripAssay® (ViennaLab Diagnostics, Vienna, Austria). In addition, all coding sequences of the *CYP21A2* gene were sequenced with the Sanger method using internal sequencing primers. For analysis, the *CYP21A2* reference sequence NM_000500.6 was used.

In vitro Cell Assays

The antiretroviral HIV drugs zidovudine (3′-azido-3′deoxythymidine) and efavirenz were purchased from Sigma-Aldrich (Buchs, Switzerland), and tenofovir and emtricitabine from Toronto Research Chemicals (Toronto, ON, Canada). Radiolabeled [^{3}H]-17OHP (50 Ci/mmol) was from American Radiolabel Chemicals Inc. (St. Louis, MO, USA). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was obtained from Sigma-Aldrich (Buchs, Switzerland). The human adrenal carcinoma cell line NCI-H295R originates from American Type Culture Collection.

NCI-H295R cells were cultured under standard conditions [12, 20]. Test drugs were dissolved in dimethyl sulfoxide (DMSO); final concentrations used for treatment were within and above the range of mean effective serum concentrations reported in use for HIV therapy. Cells were grown in 12-well plates. Drugs were added to normal growth medium for 3 and 24 h. Control cells were treated with 0.1% (v/v) DMSO. Radiolabeled [^{3}H]-17OHP (50,000 cpm/well) was added to the culture medium for the last 90 min of incubation. Steroids were then extracted from cell supernatants and separated by thin-layer chromatography as described [21]. Steroids were visualized on a Fuji PhosphoImager FLA-7000 (Fujifilm, Dielsdorf, Germany) and densitometrically quantified using Multi Gauge software (Fujifilm). The conversion (in %) of 17OHP to 11-deoxycortisol was taken as a measure of CYP21A2 activity.

The MTT cell proliferation assay was used to determine cell viability and proliferation of NCI-H295R cells treated with HIV drugs. In brief, cells were cultured on 96-well plates at a density of 15,000 cells/well in 200 µL medium. After 48 h, cells were treated with the HIV drugs for 3 and 24 h, and cell proliferation was assessed by adding 20 µL of MTT to the culture medium for 3 h before reading the absorbance at 540 nm on a Spectramax M2e microplate reader (Molecular Devices, CA, USA). In this assay, the absorbance at 540 nm correlates directly to the number of viable cells.

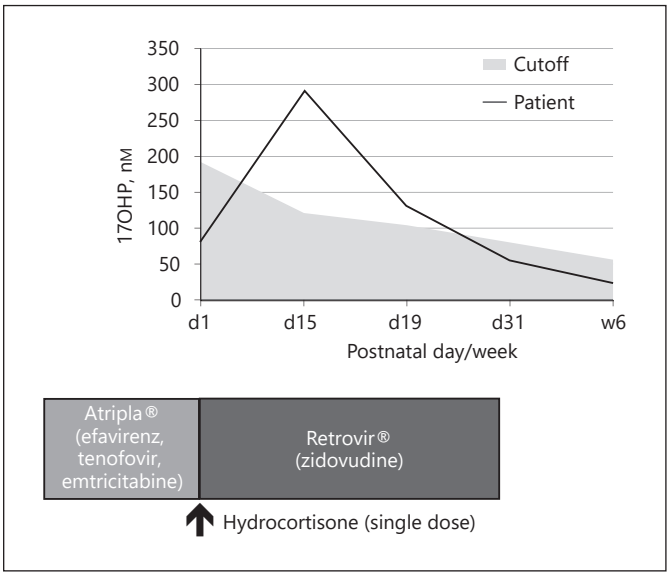

Fig. 1. Transiently elevated serum 17-hydroxyprogesterone (17OHP) levels in a newborn under the influence of Atripla® (efavirenz, tenofovir, and emtricitabine) prenatally and Retrovir® (zidovudine) during 4 weeks postnatally. Note that the newborn received 1 dose of hydrocortisone in the first 24 h after birth. Peak 17OHP levels were seen on day 15 and normalized by day 28 according to normative values of the Swiss 17OHP neonatal screening test.

In vitro Studies with Recombinant CYP21A2 Protein

Human CYP21A2 protein was expressed in *Escherichia coli* strain C43 (DE3) (Lucigen, Middleton, MI, USA) and purified via metal chelate (IMAC) and ion exchange chromatography as described [22]. Carbon monoxide difference spectroscopy was carried out for quantitative enzyme characterization following the typical absorption maximum at 450 nm with an extinction coefficient of 91 $mM^{-1} \times cm^{-1}$. Human NADPH-cytochrome P450 reductase (POR) was expressed in *E. coli* C43 (DE3) and purified by IMAC as described [23].

Inhibition studies were performed in reconstituted in vitro assays in 50 mM HEPES buffer (pH 7.4) containing 20% glycerol and 100 µM 1,2-dilauroyl-sn-glycerol-3-phosphocholine. Before use, the buffer was sonicated in a water bath for 5 min for the reconstitution of 1,2-dilauroyl-sn-glycero-3-phosphocholine vesicles. The final concentration of human CYP21A2 was 0.1 µM, and equal amounts of human POR were added. Additionally, the reaction contained an NADPH regeneration system consisting of 5 mM glucose-6-phosphate, 1 mM $MgCl_2$, and glucose-6-phosphate dehydrogenase. The steroid substrate 17OHP was added at a concentration of 5 µM, and the drugs were tested at concentrations of 5 or 50 µM. The substrate concentrations were kept below saturation but in excess of the K_m for CYP21A2. The final DMSO concentration was kept <2%. The reaction was started with 5 mM NADPH and incubated in a shaking water bath for 4–7 min at 37 °C. The reaction was quenched with chloroform. Steroids were extracted twice with chloroform, dried, and stored at –20 °C for HPLC analysis, specifically the measurement of 17OHP conversion to 11-deoxycortisol.

Steroid analysis was finally carried out by reverse phase (RP)-HPLC using a Jasco RP-HPLC system of the LC900 series (Jasco Inc, Easton, MD, USA) and a 4.6 × 125 mm NucleoDur C18 Isis RP column (Macherey-Nagel, Düren, Germany). Samples were measured within 30 min at 240 nm and a flow rate of 0.8 mL/min; solvent gradient was as follows: 80% solvent A (10% acetonitrile in water) for 13 min, 60% solvent A for 7 min, 80% solvent B (100% acetonitrile) for 2 min, and 80% solvent A for 8 min.

In silico Protein Structure Analysis

A 3D crystal structure of human CYP21A2 was obtained from the Protein Data Bank (PDB; www.rcsb.org) for docking analysis of efavirenz binding [24]. However, the structure (PDB #4Y8W) has multiple residues missing that creates gaps in the peptide backbone making it unstable during molecular-dynamic analysis. A model building using sequence conservation information and secondary structure analysis was needed to fill the gaps and create a complete structure suitable for docking and molecular-dynamic calculations. To get the secondary structure information of missing residues, we performed sequence alignments with multiple CYP21A2 protein sequences from different organisms (online suppl. Fig. 1) and made in silico calculations with the programs YASARA [25] and WHATIF [26]. Missing hydrogen atoms were added with YASARA [25], which was also used for all subsequent computations unless stated otherwise. Afterwards, the system was subjected to 500 ps explicit solvent MD simulations at 310 K, preceded by 500 steps of steepest descent and simulated annealing minimization with the AMBER15 force field and the TIP3P water model [27, 28]. All subsequent MD simulations retained these settings. The resulting minimum energy structure was used with AutoDock Vina [29] for docking experiments with efavirenz. Orthorhombic docking was grid established around the central heme. The final poses were selected based on their docking scores and resemblance to the cocrystallized progesterone in the template structure (PDB: 4Y8W). Structure models were depicted with PyMOL (www.pymol.org) and rendered as ray-traced images with POV-Ray (www.povray.org). Ligand interactions were analyzed and depicted with LigPlot$^+$ (http://www.ebi.ac.uk/thornton-srv/software/LigPlus/).

Statistical Analysis

Statistical analysis was performed with Microsoft Excel and Prism 6 software (Graph Pad Software Inc., San Diego, CA, USA). Student's *t* test was used to evaluate significant differences between values. Quantitative data represent the mean of 2 or 3 independent experiments; error bars indicate SEM. Significance was set at values of $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Results

Biochemical data of neonates exposed to the HIV drugs lopinavir and ritonavir suggested an inhibitory effect on adrenal steroidogenesis [13, 14]. Similarly, we observed elevated serum 17OHP levels and urinary steroid profile abnormalities consistent with diminished CYP21A2 activity and maybe diminished CYP17A1 activity in a preterm girl after exposure to tenofovir, efavirenz, and emtricitabine in utero, and zidovudine postnatally (see Case Report; Fig. 1 and online suppl. Table 1). Genetic testing excluded mutations in the *CYP21A2* gene.

Efavirenz Inhibits CYP21A2 Activity

The effect of the antiviral HIV drugs zidovudine, emtricitabine, tenofovir, and efavirenz on steroidogenesis was tested in the adrenal H295R cell line. Efavirenz is a nonnucleoside reverse transcriptase inhibitor. Its reported mean effective serum concentration is 1.6–9.1 μM (https://www.medicines.org.uk/emc/product/8659/smpc). The drug was tested at 4 concentrations (1, 5, 10, and 50 μM) after 3 and 24 h of incubation. A significant decrease in CYP21A2 activity was observed after 3-h incubation starting at 5 μM ($p < 0.05$), with a clear additional dose effect at higher concentrations (10 and 50 μM; $p < 0.01$, $p < 0.001$) (Fig. 2a). By contrast, after 24-h incubation, CYP21A2 inhibition was only observed at the highest (50 μM) efavirenz concentration ($p < 0.001$) suggesting that longer incubation may allow the cells to compensate for the drug effect to some degree (Fig. 2b).

Zidovudine and emtricitabine are nucleoside reverse transcriptase inhibitors. Reported mean effective serum concentrations for zidovudine are 4.45–8.5 μM (https://www.medicines.org.uk/emc/product/6811/smpc) and for emtricitabine 4.5–10 μM (https://www.medicines.org.uk/emc/product/18/smpc). H295R cells were treated with zidovudine and emtricitabine at 2 concentrations (10 and 50 μM) for 3 and 24 h. Both drugs showed no effect on CYP21A2 activity at either timing (Fig. 2). Tenofovir is also a nucleoside reverse transcriptase inhibitor, but in contrast to zidovudine and emtricitabine its mean effective serum concentration is markedly lower (0.74–1.13 μM) (https://www.medicines.org.uk/emc/product/771/smpc). Therefore, tenofovir was tested at lower concentrations (1 and 5 μM) for 3 and 24 h. No effect on CYP21A2 activity was observed (Fig. 2).

As the HIV drugs efavirenz, tenofovir, and emtricitabine are often in use as triple medication (Atripla®), we also tested the 3 compounds together in our H295R cell system. These experiments revealed the same inhibitory effect on CYP21A2 as observed with efavirenz alone (Fig. 2, bottom).

Furthermore, to assess whether the tested drugs may affect H295R cell viability, we performed an MTT cell proliferation assay. Efavirenz starting at 5 μM reduced cell proliferation significantly compared to control cells (online suppl. Fig. 2). Efavirenz at 10 and 50 μM seemed extremely cytotoxic. The same effect was also observed in

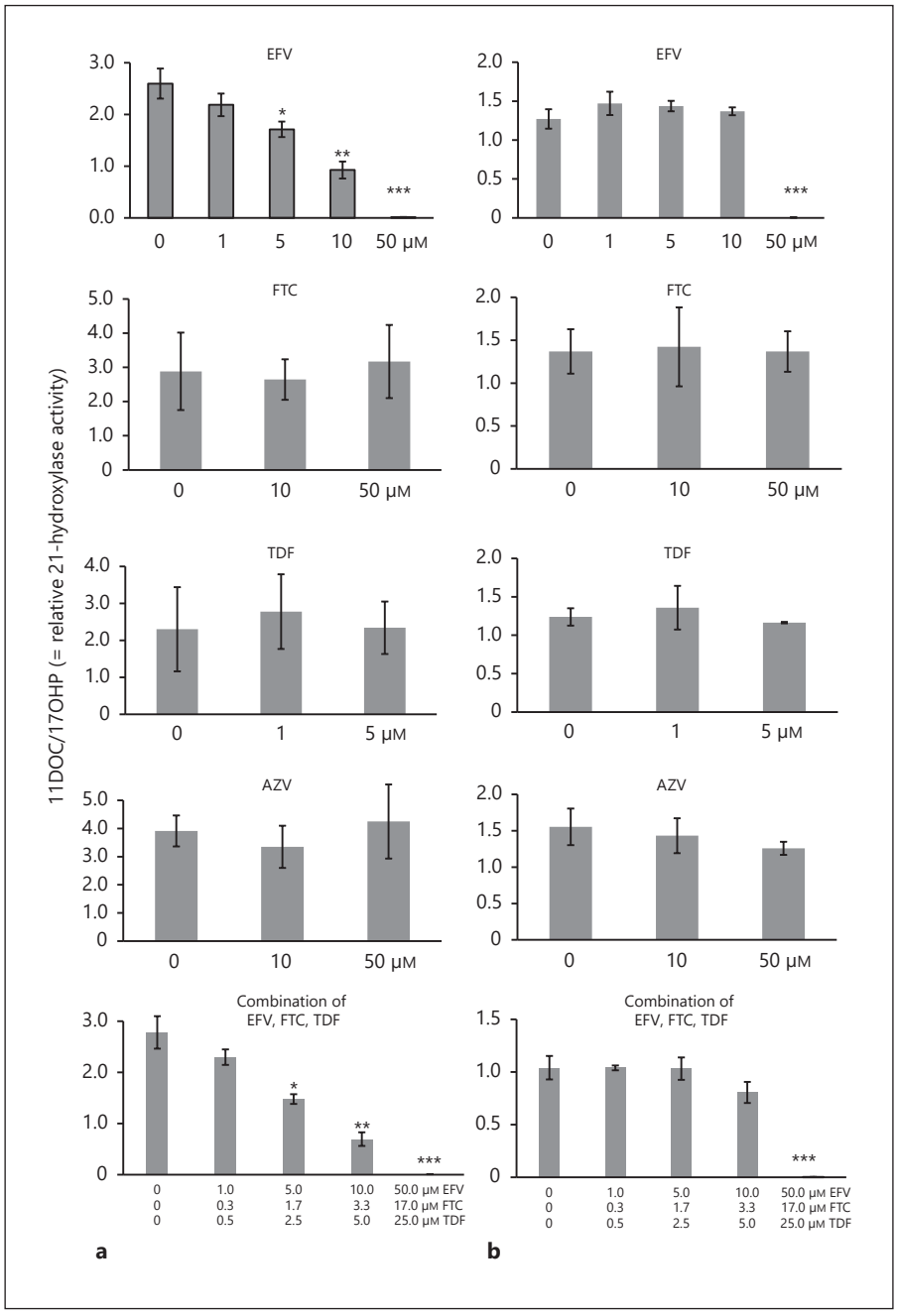

Fig. 2. Efavirenz (EFV) inhibits CYP21A2 activity at therapeutic concentrations, while no effect was seen for the other tested HIV drug compounds. Human adrenal NCI-H295R cells were treated with EFV, emtricitabine (FTC), tenofovir (TDF), and zidovudine (AZV) at various concentrations for 3 h (**a**) and 24 h (**b**) to test for their effect on steroidogenesis. Steroids were extracted from cell supernatants and separated by thin-layer chromatography. Relative CYP21A2 activity was calculated by assessing the conversion of 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol (11DOC). Data are means ± SEM of 2–4 independent experiments. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

H295R cells treated with the Atripla® combination, while no effect was seen with single treatments of zidovudine, emtricitabine, and tenofovir.

Efavirenz Is a Direct Inhibitor of CYP21A2 Activity

We also tested the direct effect of the 4 HIV drugs on enzyme catalysis using human recombinant CYP21A2 protein and 17OHP as substrate. Similar to the cell culture experiments, we found that efavirenz inhibited CYP21A2 activity to a significant degree, decreasing the product formation to 30% at a concentration of 50 μM efavirenz (Fig. 3). By contrast, no effect on enzyme activity was found for the 3 other test drugs (Fig. 3).

Computational Docking of Efavirenz into the Human CYP21A2 Crystal Structure

Efavirenz was docked into the crystal structure of human CYP21A2 using Autodock VINA (Fig. 4a). Superim-

position of CYP21A2 structures with either progesterone or efavirenz docked into the active site revealed similarities in binding poses (Fig. 4b, c). We observed a distance of 2.8 Å between efavirenz and heme iron at the active site of CYP21A2 (Fig. 4a). Efavirenz is smaller than the progesterone molecule and may achieve several potential binding poses inside CYP21A2. However, the occupation of the CYP21A2 catalytic site by efavirenz would inhibit the binding and metabolism of CYP21A2 steroid substrates. A comparison of CYP21A2 and CYP17A1 crystal structures complexed with steroid substrates and docked efavirenz into the crystal structure of CYP21A2 revealed similarities in interacting residues; the distance of the efavirenz nitrogen to the central heme iron of CYP21A2 was in similar range (Fig. 4a). Binding of efavirenz with CYP21A2 shares many similarities with natural substrates of CYP21A2 with identical active site residues such as Ser109, Gly292, and Leu364 involved in binding for both chemicals (Fig. 4b, c).

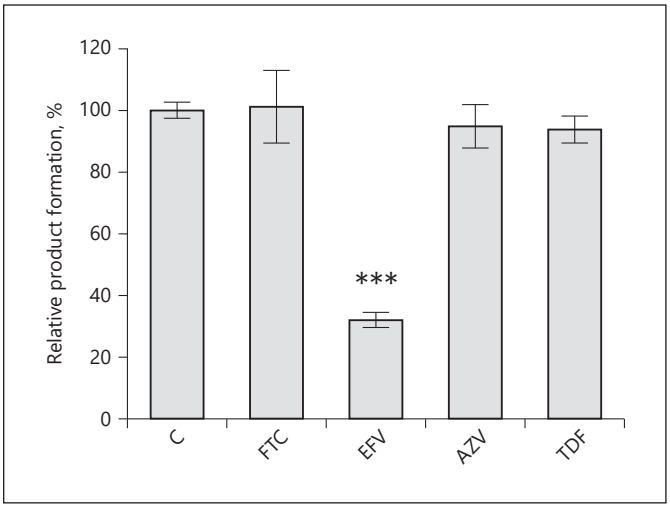

Fig. 3. Effect of HIV drugs on CYP21A2 activity using human recombinant CYP21A2 protein produced in bacteria. Protein was produced as published. CYP21A2 activity was tested with 5 μM 17-hydroxyprogesterone (17OHP) as substrate and HIV drugs at the following concentrations: emtricitabine (FTC) 50 μM, efavirenz (EFV) 50 μM, zidovudine (AZV) 50 μM, and tenofovir (TDF) 5 μM. Steroids were extracted and analyzed by RP-HPLC (see Methods). C, control. *** $p < 0.001$.

Discussion

Laboratory investigations of neonates exposed to HIV drugs suggest that these compounds may alter adrenal steroidogenesis [13, 14]. However, so far, these drugs had not been tested for their possible effect on adrenal steroidogenesis. In this study, we show that the antiretroviral drug efavirenz inhibits the steroidogenic enzyme CYP21A2 at therapeutic concentrations in human adrenal NCI-H295R cells and in direct kinetic protein interaction assays. Our bioinformatic studies suggest that efavirenz is a direct competitor to substrates of the enzyme. By contrast, no inhibitory effect on CYP21A2 activity was found for zidovudine, emtricitabine, and tenofovir.

Medications in use for HIV treatment fall into different categories concerning drug safety (https://aidsinfo.nih.gov/guidelines). Tested drugs suspected to inhibit steroidogenesis are listed in the category characterized by limited experience in pregnancy and with incomplete data on teratogenicity, toxicity, and drug interactions. In the literature, efavirenz has been linked to congenital anomalies in monkeys and humans in some studies (https://aidsinfo.nih.gov/guidelines [30, 31]), but these adverse effects were not confirmed in other studies [32, 33]. Currently, no restrictions apply to the use of efavirenz during pregnancy, neither in British nor US HIV treatment guidelines (https://www.bhiva.org/pregnancy-guidelines and https://aidsinfo.nih.gov/guidelines). Fetal exposure to tenofovir was shown to lower bone mineral content in infants compared to controls [34]. By contrast, no congenital anomalies or adverse effects have been reported with the HIV drug emtricitabine [33, 35, 36].

Concerning interactions of HIV drugs with cytochrome P450 enzymes, nothing was known for steroidogenic P450 so far. However, ritonavir has been reported to inhibit drug-metabolizing CYP2D6 and CYP3A4 enzymes [37, 38], while enzyme induction of ritonavir/lopinavir on CYP1A2, CYP2C9, and CYP2C19 activities has been observed [39]. These drugs have not been tested for their effect on CYP21A2 in this study but are currently being tested in cell experiments with preliminary results showing a marked inhibitory effect of lopinavir but not rotinavir on CYP21A2 and a smaller effect on CYP17A1.

Overall, these results suggest that HIV drugs should be carefully tested for their possible effect on P450, including steroidogenic P450 enzymes, as this may have clinical implications. Mild inhibition of adrenal steroid biosynthesis may not have consequences in healthy individuals, but it can result in the inability to sustain stressful situations in patients with severe disease when there is a high demand for stress hormones within minutes. In addition, long-term, low-grade inhibition of CYP21A2 or other steroid enzyme activities may lead to an imbalance between adrenal corticosteroids and androgens with all its consequences. This is best exemplified by late-onset CAH due

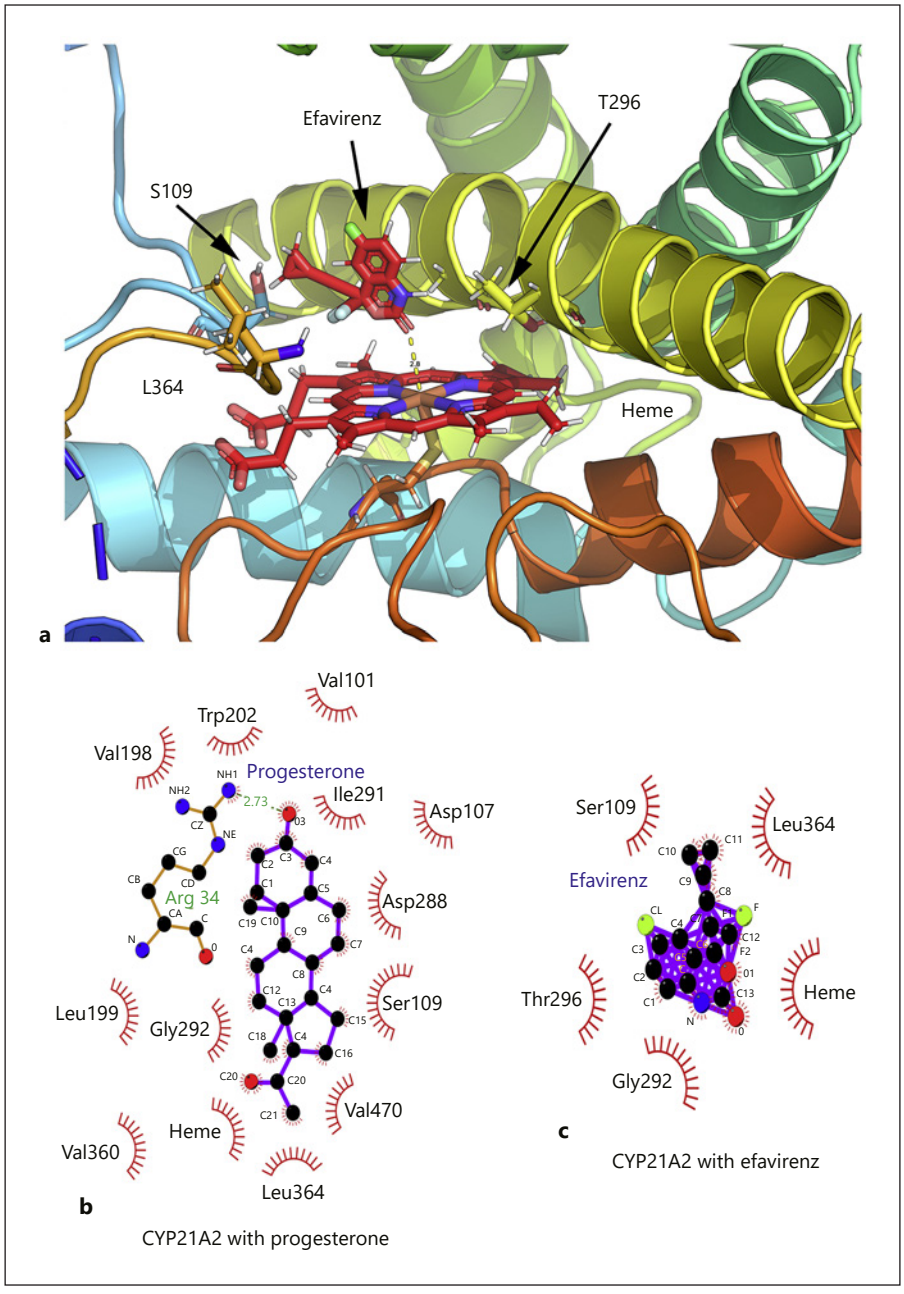

Fig. 4. Structure analysis of the interaction between the HIV drug efavirenz and the CYP21A2 enzyme. **a** Efavirenz docked into the protein structure of CYP21A2. Progesterone (**b**) versus efavirenz (**c**) binding poses revealed by the CYP21A2 structure and docking studies.

to mild CYP21A2 deficiency. Such an effect may also result from medications interfering with steroid enzyme activities, as shown for antiepileptic drugs, which can cause an increase in androgen production and a polycystic ovary syndrome phenotype [40, 41].

The premature baby described in our case report showed positive newborn screening for transient elevation in plasma 17OHP and increased urinary excretion of androgens, estrogens, and progesterones after treatment with HIV drugs during fetal life (tenofovir, efavirenz, and emtricitabine) and postnatally (zidovudine). While elevated plasma 17OHP during neonatal screening motivated us to investigate the effect of the HIV drugs in question on CYP21A2 activity, elevated urinary progesterone excretion may not be explained by CYP21A2 inhibition but rather by CYP17A1 deficiency. On the other hand, CYP17A1 deficiency decreases androgen production. Thus, the abnormal biochemical

findings in our case with spontaneous resolution may or may not have been due to an adverse drug effect (of efavirenz). However, our in vitro and in silico studies show that such an effect is clearly seen for efavirenz. Its impact should be further explored in larger clinical studies. It is therefore important to bring the possible inhibitory effect of HIV drugs on adrenal steroidogenesis to the attention of the HIV community, as it may have clinical implications beyond the neonatal age.

Conclusion

The HIV drug efavirenz inhibits CYP21A2 activity in human adrenal NCI-H295R cells at therapeutic concentrations apparently by a competitive mechanism. This effect has been suspected by laboratory abnormalities found in few newborns treated with HIV drugs. Whether this effect is of clinical relevance to the adrenal function in health and disease remains to be seen in larger clinical studies investigating children and adults during HIV drug treatment. So far, we suggest to list some HIV drugs (such as efavirenz) as medications that may cause a positive neonatal screening test for elevated 17OHP.

Acknowledgment

We thank the patient and her family for providing the data for the case report.

Statement of Ethics

Parents have given written informed consent for the Case Report. The institutional ethics committee approved the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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